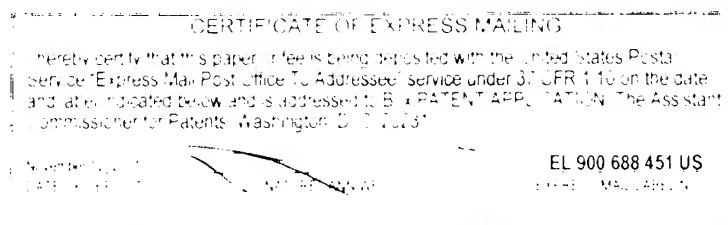


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Carol W. Readhead and Robert Winston
 Serial No. Unassigned
 Filed: November 12, 2001
 For: IN VITRO TRANSFECTION, STORAGE AND TRANSFER OF MALE
 GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES &
 GENE THERAPIES

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
 Assistant Commissioner for Patents
 Washington, D.C. 20231



Dear Sir or Madam:

This Preliminary Amendment is filed with a divisional application of pending U.S. Serial No. 09/191,920, filed November 13, 1999. The divisional application filed herewith is directed to the subject matter of Claims 1-12, as originally filed in parent U.S. Serial No. 09/191,920, which claim was designated Group IV in a restriction requirement, mailed March 24, 2000. The Examiner is respectfully requested to consider the following amendments and remarks.

AMENDMENT

A Version With Markings To Show Changes Made is included beginning at page 5, after Applicant's Remarks.

IN THE SPECIFICATION:

In the Title, at page 1, lines 1-3, please delete the entire title, and insert therefor:

--IN VITRO TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS
 FOR GENERATION OF TRANSGENIC SPECIES & GENE THERAPIES--.

At page 1, line 4, please delete the entire one-sentence paragraph, and insert the following:

--This application is a division of U.S. Non-provisional Application No. 09/191,920, filed on November 13, 1998, which claims the benefit of U.S. Provisional Application No.

60,065,825, filed on November 14, 1997. This application is also related to U.S. Serial No. _____, filed on November 12, 2001, U.S. Serial No. _____, filed on November 12, 2001, and U.S. Serial No. _____, filed on November 12, 2001, which are all divisions of U.S. Serial No. 09/191,920. This application is also related to U.S. Serial No. 09/272,443, filed March 19, 1999, which is a continuation of 09/191,920.--

At page 4, line 14 through page 15, line 1, please delete the entire paragraph, and insert therefor the following:

--This invention also relates to a novel method for the isolation of spermatogonia, comprising obtaining spermatogonia from a mixed population of testicular cells by extruding the cells from the seminiferous tubules and gentle enzymatic disaggregation. The spermatogonia or stem cells which are to be genetically modified, may be isolated from a mixed cell population by a novel method including the utilization of a promoter sequence, which is only active in cycling spermatogonia stem cell populations, for example, b-Myb or a spermatogonia specific promoter, such as the c-kit promoter region, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, or FRM1 (from fragile X site) promoter, optionally linked to a reporter construct, for example, the Green Fluorescent Protein Gene (EGFP). These unique promoter sequences drive the expression of the reporter construct only in the cycling spermatogonia. The spermatogonia, thus, are the only cells in the mixed population which will express the reporter construct and they, thus, may be isolated on this basis. In the case of the green fluorescent reporter construct, the cells may be sorted with the aid of, for example, a FAC's scanner set at the appropriate wavelength or they may be selected by chemical methods.--

At page 10, lines 11-17, please delete the entire paragraph and insert therefor the following:

--"Gene delivery (or transfection) mixture", in the context of this patent, means selected genetic material together with an appropriate vector mixed, for example, with an effective amount of lipid transfecting agent. The amount of each component of the mixture is chosen so that the transfection of a specific species of germ cell is optimized. Such optimization requires no more than routine experimentation. The ratio of DNA to lipid is